

APP- α assay and the APP- α /FL assay (Esch et al., supra). The brain homogenate samples in 5 M guanidine were diluted 1:10 in ELISA Specimen Diluent (0.014 M phosphate buffer, pH 7.4, 0.6% bovine serum albumin, 0.05% thimerosal, 0.5 M NaCl, 0.1% NP40). They were then diluted 1:4 in Specimen Diluent containing 0.5 M guanidine. Diluted homogenates were then centrifuged at 16,000 x g for 15 seconds at RT. The APP standards and samples were added to the plate in duplicate aliquots and incubated for 1.5 hr at RT. The biotinylated reporter antibody 2H3 or 16H9 was incubated with samples for 1 hr at RT. Streptavidin-alkaline phosphatase (Boehringer Mannheim), diluted 1:1000 in specimen diluent, was incubated in the wells for 1 hr at RT. The fluorescent substrate 4-methyl-umbelliperyl-phosphate was added for a 30-min RT incubation and the plates were read on a Cytofluor tm 2350 fluorimeter (Millipore) at 365 nm excitation and 450 nm emission.

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 and 2, after page 87.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a paper copy containing the above named sequences, SEQ ID NOS:1-5. The paper copy of the sequence information has been printed from the floppy disk filed in Application No. 09/322,289.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 326-2400.

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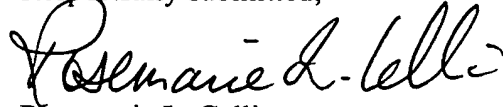
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SCHENK, Dale B.
Application No.: 09/723,713
Page 4

PATENT

Respectfully submitted,

A handwritten signature in cursive script, reading "Rosemarie L. Celli".

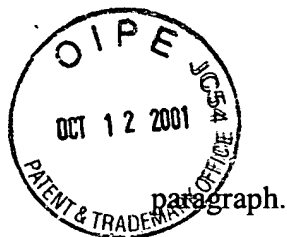
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please replace the paragraph beginning at line 23 of page 11 with the following

H2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH. (SEQ ID NO:1)

Please replace the paragraph beginning at line 2 of page 50 with the following paragraph.

Aβ1-12 peptide	NH2-DAEFRHDSGYEV <u>C</u> -COOH (<u>SEQ ID NO:2</u>)
Aβ1-5 peptide	NH2-DAEFR <u>C</u> -COOH (<u>SEQ ID NO:3</u>)
Aβ33-42 peptide	NH2- <u>C</u> -amino-heptanoic acid-GLMVGGVVIA-COOH (<u>SEQ ID NO:4</u>)
Aβ13-28 peptide	Ac-NH-HHQLVFFAEDVGSNK <u>GGC</u> -COOH (<u>SEQ ID NO:5</u>)

Please replace the paragraph beginning at line 30 of page 83 with the following paragraph.

Two different APP assays were utilized. The first, designated APP-α/FL, recognizes both APP-alpha (α) and full-length (FL) forms of APP. The second assay is specific for APP-α. The [APP-α/FL]APP-α/F assay recognizes secreted APP including the first 12 amino acids of Aβ. Since the reporter antibody (2H3) is not specific to the α-clip-site, occurring between amino acids 612-613 of APP695 (Esch et al., Science 248, 1122-1124 (1990)); this assay also recognizes full length APP (APP-FL). Preliminary experiments using immobilized APP antibodies to the cytoplasmic tail of APP-FL to deplete brain homogenates of APP-FL suggest that approximately 30-40% of the APP-α /FL APP is FL (data not shown). The capture antibody for both the APP-α/FL and APP-α assays is mAb 8E5, raised against amino acids 444 to 592 of the APP695 form (Games et al., supra). The reporter mAb for the APP-α/FL assay is mAb 2H3, specific for amino acids 597-608 of APP695 (Johnson-Wood et al., supra) and the reporter antibody for the APP-α assay is a biotinylated derivative of mAb 16H9, raised to amino acids 605 to 611 of APP. The lower limit of sensitivity of the APP-αFL assay is about 11 ng/ml (150 pM) (Johnson-Wood et al.) and that of the APP-α specific assay is 22 ng/ml (0.3 nM). For both APP assays, mAb 8E5 was coated onto the wells of 96-well ELA plates as described above for

mAb 266. Purified, recombinant secreted APP- α was used as the reference standard for the APP- α assay and the APP- α /FL assay (Esch et al., supra). The brain homogenate samples in 5 M guanidine were diluted 1:10 in ELISA Specimen Diluent (0.014 M phosphate buffer, pH 7.4, 0.6% bovine serum albumin, 0.05% thimerosal, 0.5 M NaCl, 0.1% NP40). They were then diluted 1:4 in Specimen Diluent containing 0.5 M guanidine. Diluted homogenates were then centrifuged at 16,000 x g for 15 seconds at RT. The APP standards and samples were added to the plate in duplicate aliquots and incubated for 1.5 hr at RT. The biotinylated reporter antibody 2H3 or 16H9 was incubated with samples for 1 hr at RT. Streptavidin-alkaline phosphatase (Boehringer Mannheim), diluted 1:1000 in specimen diluent, was incubated in the wells for 1 hr at RT. The fluorescent substrate 4-methyl-umbelliperyl-phosphate was added for a 30-min RT incubation and the plates were read on a Cytofluor tm 2350 fluorimeter (Millipore) at 365 nm excitation and 450 nm emission.